Sporethanoligenium mesophilum gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BM^T is GU645013.

Summary

A novel mesophilic, strictly anaerobic bacterium, strain BM^T was isolated from food industry wastewater. The cells were long, motile, spore-forming rod and stained Gram-negative. Growth of strain BM^T was observed at 16-44 ºC (optimum 37 ºC) and pH 6.0-9.0 (optimum 7.5). The salinity range for grow was 0-8% g (optimum 1.5 g) NaCl l^{-1}. Strain BM^T was chemo-organotrophic and could use a few sugars and amino acids as carbon and energy sources. The fermentation products from peptone-yeast broth were propionate, acetate, ethanol and isovalerate. Indole, NH_3 and H_2S were produced from peptone. Sulfur and sulfate could be used as electron acceptor. The major fatty acids detected were iso-$C_{15:0}$ (33.7%), iso-$C_{14:0}$ 3-OH (21.8%), $C_{14:0}$ (6.3%), iso-$C_{13:0}$ (4.2%), and $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ I/H (4.1%). The DNA G+C content was 28.2 mol%. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain BM^T was closely related with different genus belonging to the first family, *Clostridiaceae*, of the *Clostridiales* with the highest 16S rRNA gene sequence similarity to The three nearest published species were (with pairwise similarity values in the brackets) *Sporosalibacterium faouarense* DSM 21485^T (94.4%), *Clostridiisalibacter paucivorans* JCM 14354^T (91.9%) and *Proteiniborus ethanoligenes* JCM 14574^T (91.8%). Due to its phenotypic and genotypic characteristics, isolate BM^T is proposed as a novel species of a new genus, *Sporethanoligenium mesophilum* gen. nov., sp. nov. The type strain is BM^T (=JCM 16868^T=CGMCC)

Zhacai is a kind of preserved vegetable which has been favored in China since it was originally salted during the 1890s. The tumid stem of *Brassica juncea* var. *tumida* was generally used to make the juicy, salty and a little bit sour pickle. During the salting process, microorganisms that consist of bacteria, yeasts and fungi take important roles in degradation of protein, cellulose and starch of the vegetable to form the distinctive flavor.

The industrial manufacturing technology of zhacai was introduced to China during the 1980s, accompanied by industrial wastewater with high salinity (2%-8%) and organic load. The microbiological degradation was proved to be an effective technology to treat the industrial wastewater. When developing the technology, the microbiological composition of the wastewater was investigated and several strains with low 16S rRNA gene sequence similarity to known species were isolated, such as *Citricoccus zhacaiensis* (Meng *et al*., 2010), TY (92.3% with *Pectinatus cerevisiiphilus*, not published) and YJ1 (89.8% with *Ruminococcus gnavus*, not published). Here, we report a novel species of a novel genus belonging to the first family *Clostridiaceae* within the order *Clostridiale* (Wiegel, 2009), which is strictly anaerobic, mesophilic, spore-forming bacterium, isolated from food industry wastewater from a factory in Cixi, Zhejiang province, China.

The DSMZ medium 104b was used for isolation and cultivation. The wastewater was serially diluted and inoculated into Hungate roll-tube (Hungate, 1969) under O_2-free N_2. 5ml DSMZ medium 104b was distributed into each Hungate tubes, to solidify the medium, 1.5% (w/v) agar was added. Hungate roll-tube technique was performed several times to acquire the pure culture of strain BM^T.

The morphology of the cell were examined under optical (BX 40, Olympus) and electron (JEM-1230, JEOL) microscope. The Gram staining was performed in all grow phases using *Escherichia coli* as negative control and *Staphylococcus aureus* as positive control. Silver-plating staining was performed to observe the flagellum which was also observed under electron microscope. The bacterial cells prepared for the

electron microscope studies were collected from the PY plate which was incubated in the anaerobic chamber (Bugbox, Ruskinn) for 72 h. The ultrathin section was performed to acquire the ultrastructure of the cell.

Strain BM^T was a long, thin rod (0.3-0.6 μm × 2.0-9.2 μm) with monotrichous flagellum (Fig. 1a). Cells grew singly or in pairs. The cells of strain BM^T stained Gram-negative in all growth phases and the ultrastructure also revealed a Gram-negative-type cell wall (Fig. 1b). Spherical and terminal spores were formed in late-exponential and stationary phases (Fig. 1c).

The growth conditions for strain BM^T were determined in DSMZ medium 104b. All the experiments were performed in triplicate. The temperature range for growth was determined using water bath between 10 to 60 ºC at 1 ºC intervals. To study the NaCl requirements, NaCl in DSMZ medium 104b was removed and additional NaCl was added at the concentration (g l^{-1}) of 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150 and 200. To examine pH range for growth, the medium was adjusted to the desired pH using sterile solutions (10%) of HCl or NaOH and MES (pH 5.5-6.0), PIPES (pH 6.5-7.0), Tricine (pH 7.5-8.5), CAPSO (pH 9.0-9.5) or CAPS (10.0-11.5) were added at a concentration of 25 mM.

Strain BM^T was strictly anaerobe of which the growth was inhibited by traces of oxygen in the medium (as indicated by the pink colour of resazurin). The optimal temperature for strain BM^T was 37 ºC (range 16-44 ºC). NaCl was not necessary for growth for its range (l^{-1}) of 0-80 g with an optimum of 15 g. The pH range for growth was 6.0-9.0 with an optimum of 7.5, no growth was observed under 5.5 or above 9.5.

Substrate utilization was studied in the basal medium contained (l^{-1}): 0.3 g KH_2PO_4, 0.3 g K_2HPO_4, 1.0 g NH_4Cl, 15 g NaCl, 0.1 g KCl, 0.1 g $CaCl_2.2H_2O$, 0.1 g $MgCl_2.6H_2O$ and 1 ml 0.1% (w/v) resazurin. The pH was adjusted to 7.5. Sugar (20 mM), alcohol (0.1 %), organic acid and amino acid (20 mM) were added into basal medium in the presence of 0.1 g yeast extract l^{-1}. Growth on yeast extract (BD), peptone (BD), tryptone (BD), and casamino acids (BD) were also examined. The

fermentation products were analyzed using HPLC with an ion exclusion column (Aminex hpx-87h, BioRad). To study potential electron acceptors, elemental sulfur (0.1%, w/v), sulfate (20mM), thiosulfate (20mM), nitrate (20mM) and nitrite(20mM) were added into growth medium. The methyl red and Voges-Proskauer reactions, H_2S and indole production and catalase and oxidase activity were determined as Wu *et al.* (2009) described.

Strain BM^T grew heterotrophically on yeast extract, peptone, tryptone and casamino acids. The fermentation products on peptone-yeast broth were propionate, acetate, ethanol and isovalerate. Strain BM^T could use four amino acids as sole carbon and energy sources: glutamic acid, glycine, methionine and valine. Formate, acetate and ethanol were formed from all the four amino acids. Fructose, glucose, ribose, sucrose and pyruvate were used; acetate and ethanol were produced from these substrates. The following substrates are not used: alanine, arginine, asparagine, aspartate, cysteine, glutamine, histidine, isoleucine, leucine, lysine, phenylalamine, proline, serine, threonine, trptophan, tyrosine, arabinose, cellobiose, galactose, lactose, maltose, mannose, melibiose, raffinose, rhamnose, salicin, sorbose, starch, trehalose, xylose, inositol, mannitol, sorbitol, methanol, ethanol, propanol, citrate, fumarate, malate, succinate, malonate, formate, acetate, propionate, lactate, cellulose or xylan. Although tryptophan was not used as sole carbon source, indole was produced from peptone. Elemental sulfur and sulfate could enhance growth and biomass but not thiosulfate, nitrate or nitrite.

Fatty acids methyl esters (FAMEs) were obtained from freeze-dried cells as described by Kuykendall et al. (1988) after cultivation in DSMZ medium 104b at 37 ºC for 48h. Identification and quantification of the FAMEs were automatically performed by the Sherlock Microbial Identification System with the standard MIS Library Generation Software (Microbial ID Inc., Newark, Delaware). The main fatty acids of strain BM^T were iso-$C_{15:0}$ (33.7%), iso-$C_{14:0}$ 3-OH (21.8%), $C_{14:0}$ (6.3%), iso-$C_{13:0}$ (4.2%), and $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ I/H (4.1%), plus one major content remained undefined (unknow 13.565, 8.2%). Other fatty acids of less proportion were also found (Table

2).

Genomic DNA was extracted and the 16S rRNA gene was amplified as Wu *et al.* (2010) described. The DNA G+C content determined by reverse-phase HPLC according to Mesbah (1989) was 28.2 mol%. An almost complete 16S rRNA sequence (1490 nucleotides) was compared with closely related species with EzTaxon service (Chun *et al.*, 2007). Sequence data were aligned with clustal w version 1.8 (Thompson *et al.*, 1994). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with MEGA 4 (Tamura *et al.*, 2007), maximum-likelihood method with PHYLIP version 3.6 (Felsenstein, 1993) were used to construct the phylogenetic tree. Evolutionary distances were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1980) for the neighbour-joining method. The neighbour-joining tree is shown in Fig.2. Its topology was also supported by the results of maximum-parsimony method and maximum-likelihood method.

Comparisons of 16S rRNA gene sequences revealed that strain BM^T belonged to the cluster XII of the *Clostridiales* (Collins *et al.*, 1994). In the latest edition (2nd edition) of Bergey's Manual of Systematic Bacteriology, strain BM^T should be placed under the family *Clostridiaceae* within the order *Clostridiales* (Rezgui *et al.*, 2010) and probably represents a novel species and a novel genus. The three nearest published species were (with pairwise similarity values in the brackets) *Sporosalibacterium faouarense* DSM 21485^T (94.4%), *Clostridiisalibacter paucivorans* JCM 14354^T (91.9%) and *Proteiniborus ethanoligenes* JCM 14574^T (91.8%). Strain BM^T was also related to two thermophilic species, *Thermohalobacter berrensis* CNCM 105955^T (91.3%) and *Caloranaerobacter azorensis* DSM 13643^T (91.1%).

Genetic and physiological traits support the fact that strain BM^T were distinct from its phylogenetical relatives (Table 1). Strain BM^T was differed from *Thermohalobacter berrensis* CNCM 105955^T (Cayol *et al*., 2000) and *Caloranaerobacter azorensis* DSM 13643^T (Wery *et al*., 2001) for that these two strains were thermophilic, with the optimal growth temperature of 65 ºC whereas the upper limiting temperature for the

161 growth of strain BM^T was 44 ºC. Additionally, strain BM^T was spore-forming species
162 while these two species were non-sporulated.
163 Moreover, strain BM^T was distinct from *Proteiniborus ethanoligenes* JCM 14574^T
164 (Niu *et al*., 2008). *P. ethanoligenes* JCM 14574^T could use no sugars or amino acid as
165 carbon and energy source while strain BM^T could ferment on four amino acids and
166 four sugars. Also *P. ethanoligenes* JCM 14574^T did not form spore whereas strain
167 BM^T did. Furthermore, the G+C content of *P. ethanoligenes* JCM 14574^T (38.0%) was
168 significantly higher than it of strain BM^T.
169 Thirdly, strain BM^T was metabolically and phenotypically differed from
170 *Clostridiisalibacter paucivorans* JCM 14354^T (Liebgott *et al*., 2008). In contrast to *C.*
171 *paucivorans* JCM 14354^T, strain BM^T could use four sugars as carbon and energy
172 source, and the utilization of amino acid were different: strain BM^T was able to use
173 glutamic acid, glycine, methionine and valine while *C. paucivorans* JCM 14354^T
174 fermented cysteine, lysine, serine and valine. In addition, strain BM^T was motile by
175 monotrichous flagellum while *C. paucivorans* JCM 14354^T was peritrichous.
176 Finally, strain BMT was distinct from the nearest phylogenetical relative
177 *Sporosalibacterium faouarense* DSM 21485^T. Firstly, the optimal growth conditions
178 for the two strains were different. The *S. faouarense* DSM 21485^T was slightly
179 thermotolerant (range 20-48 ºC) with an optimum at 40 ºC, while the mesophilic
180 strain BM^T could only grow up to 44 ºC, with its optimum at 37 ºC. *S. faouarense*
181 DSM 21485^T could tolerate high NaCl concentration (0.5-150 g l^{-1}) while the growth
182 of strain BM^T could not be observed with the concentration higher than 80 g l^{-1}.
183 Also, NaCl was necessary for *S. faouarense* DSM 21485^T whereas strain BM^T could
184 grow in the medium without NaCl. Secondly, the two strains differed in the utilization
185 of substrates as carbon and energy sources. Unlike *S. faouarense* DSM 21485^T, strain
186 BM^T was able to ferment amino acid (glutamic acid, glycine, methionine and valine)
187 and two more sugars (ribose and sucrose), and the fermentation process did not need
188 yeast extract (2.0 g l^{-1}). Furthermore, cells of strain BM^T stained Gram-negative while
189 *S. faouarense* DSM 21485^T stained Gram-positive. And the DNA G+C content of *S.*
190 *faouarense* DSM 21485^T (30.7%) was higher than the value of strain BM^T. Finally, the

cellular fatty acid contents of the two strains were different. Although the two main component of strain BM^T were the same with that of *S. faouarense* DSM 21485^T, strain BMT had a significantly lower proportion of iso-$C_{15:0}$ (33.7%) than *S. faouarense* DSM 21485^T (41.0%) did. And other differences in cellular fatty acids were concluded in Table 2.

On the basis of the genotypic and phenotypic characteristics reported above, we propose that strain BMT represents a novel species of a new genus, with the name *Sporethanoligenium mesophilum* gen. nov., sp. nov.

Description of *Sporethanoligenium* gen. nov.

Sporethanoligenium (Spo.re.tha.no.li.ge'ni.um. Gr. fem.n. *spora* a seed, in bacteriology a spore; N.L. n. *ethanol* ethanol; Gr. v. *gennao* to produce; N.L. neut. n. *Sporethanoligenium* spored ethanol producer).

Motile, rod-shaped spore-forming bacterium. Stained Gram-negative. Mesophilic and strictly anaerobic. Chemo-organotroph able to grow on yeast extract, peptone, tryptone, and Casamino acids. Ferment on a few sugars and amino acids. Sulfur and sulfate can be used as electron acceptor. The major fatty acids are iso-$C_{15:0}$ (33.7%), iso-$C_{14:0}$ 3-OH (21.8%), $C_{14:0}$ (6.3%), iso-$C_{13:0}$ (4.2%), and $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ I/H (4.1%). The DNA G+C content of the known strain is 28.2%. 16S rRNA gene sequence comparisons locate *Sporethanoligenium* in the lineage of low-G+C-content Gram-positive bacteria, in the *Clostridiaceae*, the first family of the order *Clostridiales*. The type species is *Sporethanoligenium mesophilum*.

Description of *Sporethanoligenium mesophilum* sp. nov.

Sporethanoligenium mesophilum (me.so.phi'l.um. Gr. adj. *mesos*, middle; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*), friend, loving; N.L. neut. adj. *mesophilum*, middle (temperature) -loving, mesophilic).

Cells are strictly anaerobic rods (0.3-0.6 μm × 2.0-9.2 μm), occurring singly or in pairs. Motile and monotrichous. Celles stain Gram-negative. Spores are formed in

late-exponential and stationary phases. Growth occurs between 16 and 44 ºC (optimum 37 ºC) and at pH 6.0-9.0 (optimum 7.5). The NaCl range for growth is 0-80 g (l^{-1}) with an optimum of 15 g. The major fatty acids detected are iso-$C_{15:0}$ (33.7%), iso-$C_{14:0}$ 3-OH (21.8%), $C_{14:0}$ (6.3%), iso-$C_{13:0}$ (4.2%), and $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ I/H (4.1%), and one major content remained undefined (unknow 13.565, 8.2%). Heterotrophic. Grows on yeast extract, peptone, tryptone, and casamino acids. Cells are able to ferment the following substrates in the presence of yeast extract (0.1 g l^{-1}): glutamic acid, glycine, methionine, valine, fructose, glucose, ribose, sucrose and pyruvate. Elemental sulfur and sulfate are used as electron acceptor but not thiosulfate, nitrate, or nitrite. Methyl red and Voges-Proskauer tests are negative. Catalase and oxidase activity are negative. H_2S, NH_3, and indole are produced from peptone. The DNA G+C content of the type strain is 28.2 mol%.

The type strain, strain BM^T (=JCM 16868^T=CGMCC), was isolated from food industry wastewater from a factory in Cixi, Zhejiang province, China.

Acknowledgements

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References

Cayol, J. L., Ducerf, S., Patel, B. K., Garcia, J. L., Thomas, P. & Ollivier, B. (2000). *Thermohalobacter berrensis* gen. nov., sp. nov., a thermophilic, strictly halophilic bacterium from a solar saltern. *Int J Syst Evol Microbiol* **50 Pt 2**, 559-564.

Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. & Farrow, J. A. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**, 812-826.

Felsenstein, J. (1993). PHYLIP (phylogeny inference package) version 3.5.1. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406-416.

Hungate, R. E. (1969). A roll tube method for the cultivation of strict anaerobes. In *Methods in Microbiology*, vol. 3b, pp. 117-132. Edited by J. R. N. R. W. Ribbons. London: Academic Press.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111-120.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 38, 358-361.

Liebgott, P. P., Joseph, M., Fardeau, M. L., Cayol, J. L., Falsen, E., Chamkh, F., Qatibi, A. I. & Labat, M. (2008). *Clostridiisalibacter paucivorans* gen. nov., sp. nov., a novel moderately halophilic bacterium isolated from olive mill wastewater. *Int J Syst Evol Microbiol* **58**, 61-67.

Meng, F.-X., Yang, X.-C., Yu, P.-S., Pan, J.-M., Wang, C.-S., Xu, X.-W. & Wu, M. (2010). *Citricoccus zhacaiensis* sp. nov., isolated from a bioreactor for saline wastewater treatment. *Int J Syst Evol Microbiol* **60**, 495-499.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297-306.

Niu, L., Song, L. & Dong, X. (2008). *Proteiniborus ethanoligenes* gen. nov., sp. nov., an anaerobic protein-utilizing bacterium. *Int J Syst Evol Microbiol* **58**, 12-16.

Rezgui, R., Ben Ali Gam, Z., Ben Hamed, S., Fardeau, M. L., Cayol, J. L., Maaroufi, A. & Labat, M. (2010). *Sporosalibacterium faouarense* gen. nov., sp. nov., a novel moderately halophilic bacterium isolated from an oil-contaminated soil of south Tunisia. *Int J Syst Evol Microbiol*.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596-1599.

Wery, N., Moricet, J. M., Cueff, V., Jean, J., Pignet, P., Lesongeur, F., Cambon-Bonavita, M. A. & Barbier, G. (2001). *Caloranaerobacter azorensis* gen. nov., sp. nov., an anaerobic thermophilic bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **51**, 1789-1796.

Wiegel, J. (2009). Family I. *Clostridiaceae* Prikram 1933, 90 AL. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 3, pp. 736-738. Edited by P. D. Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwing, F. A. Rainey, K. H. Schleifer & W. B. Whitman. New York: Springer.

Wu, X.-Y., Zheng, G., Zhang, W.-W., Xu, X.-W., Wu, M. & Zhu, X.-F. (2009). *Amphibacillus jilinensis* sp. nov., facultatively anaerobic and alkaliphilic bacilli from a soda lake in China. *Int J Syst Evol Microbiol*, ijs.0.018259-018250.

Wu, X.-Y., Shi, K.-L., Xu, X.-W., Wu, M., Oren, A. & Zhu, X.-F. (2010). *Alkaliphilus halophilus* sp. nov., a novel strictly anaerobic and halophilic bacterium isolated from a saline lake in China. *Int J Syst Evol Microbiol*,

ijs.0.014084-014080.

Legends to table and figure:

Table 1. Characteristics for distinguishing strain BM^T from phylogenetically related species.

Taxa: 1, Strain BM^T; 2, *Sporosalibacterium faouarense* DSM 21485^T (Rezgui *et al*., 2010); 3, *Proteiniborus ethanoligenes* JCM 14574^T (Niu *et al*., 2008); 4, *Clostridiisalibacter paucivorans* JCM 14354^T (Liebgott *et al*., 2008).Symbols: +, positive; -, negative; ND, not determined; N/A, not applicable.

Table 2. Cellular fatty acids content of strain BM^T and *Sporosalibacterium faouarense* DSM 21485^T.

Taxa: 1, strain BM^T; 2, *Sporosalibacterium faouarense* DSM 21485^T (Rezgui *et al*., 2010). Values are percentages of total fatty acids.

Fig. 1. Transmission electron micrograph of strain BM^T, showing a) monotrichous flagellum, b) Gram-negative cell wall structure, and c) terminal spore. ol

Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain BM^T and related species. Bootstrap values (only >70 % are shown) based on 1000 replications are listed as percentages at branching points. Although the bootstrap value of the branch containing strain BM^T and two other species (*Sporosalibacterium faouarense* and *Clostridiisalibacter paucivorans*) is low, it is clearly shown that these three strains are mutually closer than the two other species (*Thermohalobacter berrensis* and *Caloranaerobacter azorensis*) belonging to the adjacent branch.Bar, 0.02 substitutions per nucleotide position.

329 Table 1

Characteristic	**1**	**2**	**3**	**4**
Inhabiting niche	Food industry wastewater	Hydrocarbon-polluted soil	UASB sludge	Olive mill wastewater
Width × length (μm)	0.3-0.6 × 2.0-9.2	0.5 ×5.0-10.0	0.5-0.6 × 1.4-3.8	0.5 × 3.0-8.0
Motility	+	+	-	+
Gram type	-	+	+	+
Spore formation	+	+	-	+
Type of flagella	monotrichous	monotrichous	N/A	peritrichous
Temperature for growth (°C):				
Range	16-44	20-48	20-48	20-50
Optimum	37	40	37	42
pH for growth				
Range	6.0-9.0	6.2-8.1	6.4-10.0	5.5-8.5
Optimum	7.5	6.9	8.5-8.8	6.8
NaCl concentration for growth (g l^{-1})				
Range	0-80	5-150	0-20	10-100
Optimum	15	40	ND	50
G+C content of DNA (mol%)	28.2	30.7	38.0	33.0
Casamino acids	+	-	+	+
Substrates used				
Cysteine	-	-	-	+
Glutamic acid	+	-	ND	-
Glycine	+	-	ND	-
Lysine	-	-	-	+
Methionine	+	-	-	-
Serine	-	-	-	+
Valine	+	-	-	+
Fructose	+	+	-	-
Glucose	+	+	-	-
Ribose	+	-	-	-
Sucrose	+	-	-	-
Fumarate	-	+	-	+
Succinate	-	-	-	+
Pyruvate	+	+	-	+

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331

332 Table. 2

Fatty acids	**1**	**2**
iso-$C_{11:0}$	0.3	0.3
unknow 11.543	1.4	nd
iso-$C_{13:0}$	4.2	4.4
anteiso-$C_{13:0}$	0.6	0.4
iso-$C_{13:0}$ 3-OH	0.3	nd
$C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ I/H	4.1	0.9
$C_{13:1}$ AT 12-13	1.4	nd
unknow 13.565	8.2	nd
$C_{14:0}$	6.3	0.9
$C_{14:0}$ 2-OH	3.0	1.5
iso-$C_{14:0}$ 3-OH	21.8	21.6
iso-$C_{14:1}$	nd	0.6
unknown 14.959	1.2	nd
iso-$C_{15:0}$	33.7	41
anteiso-$C_{15:0}$	3.2	3.9
iso-$C_{15:1}$ F	1.6	2.8
$C_{15:1}$ ω5c	0.4	0.8
$C_{15:1}$ ω8c	0.6	nd
$C_{16:0}$	1.2	1.2
$C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH	1.6	nd
anteiso-$C_{17:0}$	0.8	nd
C_{17}cyc	0.4	0.6
$C_{17:1}$ ω9c	0.7	nd
iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B	2.5	1.5
iso-$C_{18:0}$	nd	1.3
anteiso-$C_{18:0}$ and/or $C_{18:2}$ ω6,9c	0.5	nd
iso-$C_{19:1}$ I	0.4	1.0

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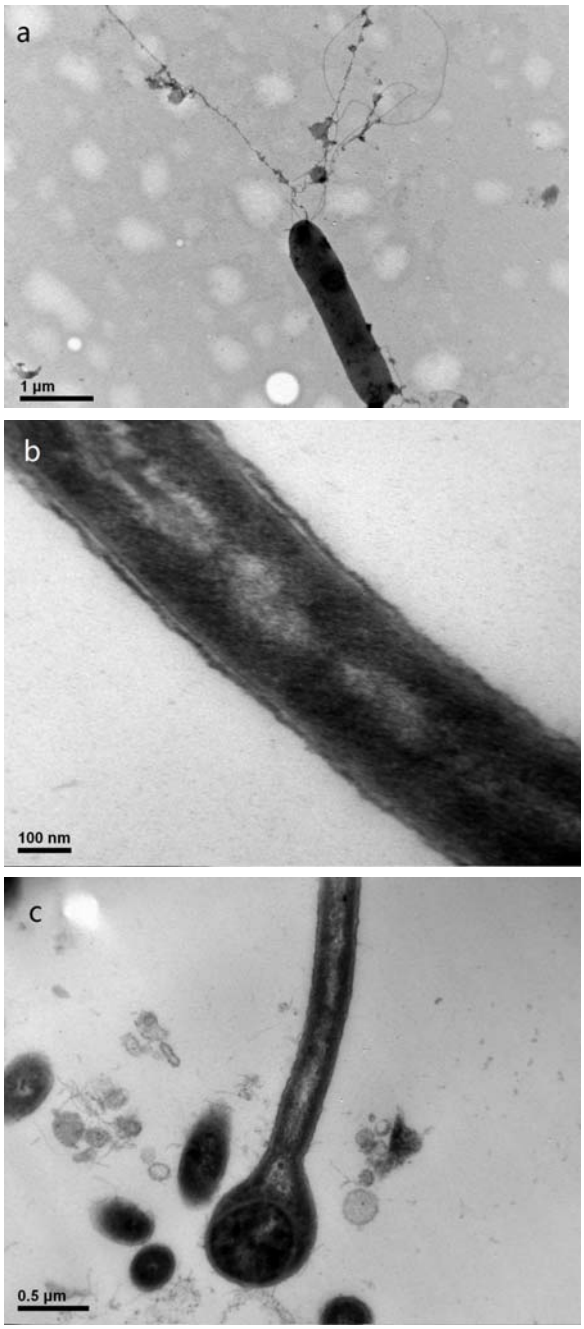

Fig. 1

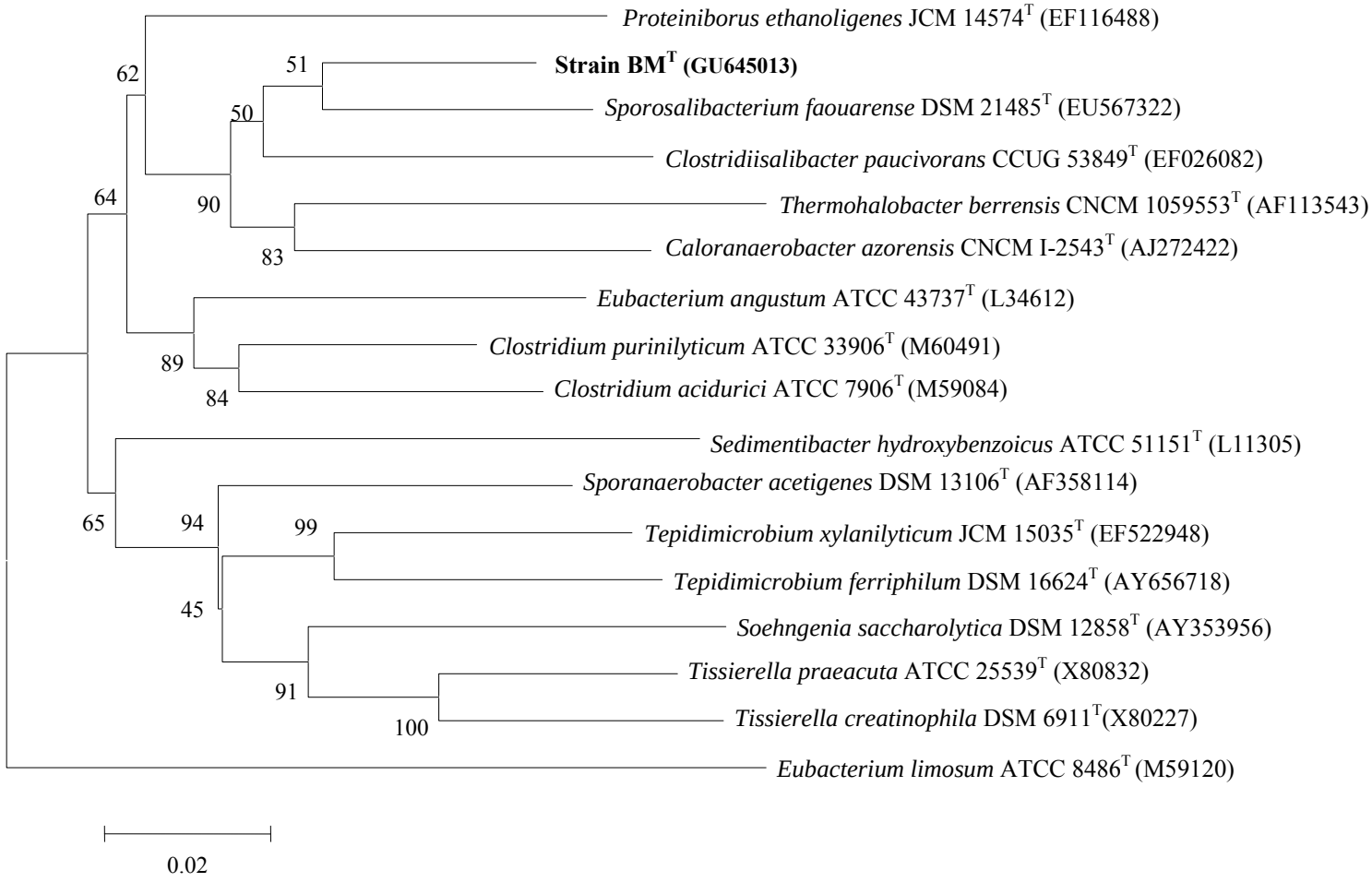
Proteiniborus ethanoligenes JCM 14574T (EF116488)
Strain BMT (GU645013)
Sporosalibacterium faouarense DSM 21485T (EU567322)
Clostridiisalibacter paucivorans CCUG 53849T (EF026082)
Thermohalobacter berrensis CNCM 1059553T (AF113543)
Caloranaerobacter azorensis CNCM I-2543T (AJ272422)
Eubacterium angustum ATCC 43737T (L34612)
Clostridium purinilyticum ATCC 33906T (M60491)
Clostridium acidurici ATCC 7906T (M59084)
Sedimentibacter hydroxybenzoicus ATCC 51151T (L11305)
Sporanaerobacter acetigenes DSM 13106T (AF358114)
Tepidimicrobium xylanilyticum JCM 15035T (EF522948)
Tepidimicrobium ferriphilum DSM 16624T (AY656718)
Soehngenia saccharolytica DSM 12858T (AY353956)
Tissierella praeacuta ATCC 25539T (X80832)
Tissierella creatinophila DSM 6911T(X80227)
Eubacterium limosum ATCC 8486T (M59120)
62
51
50
64
90
83
89
84
65
94
99
45
91
100
0.02

Fig. 2